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Chemotherapy with hybrid liposomes for lymphoma without drugs in vivo

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Abstract

Hybrid liposomes composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene (*n*) dodecyl ether ($C_{12}(EO)_n$, $n = 21$ and 25) were prepared with the method of sonication. Clear solution of hybrid liposomes having hydrodynamic diameter of 80–100 nm could be maintained over 3 weeks. Hybrid liposomes induced apoptosis for human lymphoma (MOLT-4 and RAJI) cells in vitro. No toxicity was observed in the rats after intravenously injecting hybrid liposomes in vivo. We clearly demonstrated that a mouse model of lymphoma was established and prolonged survival was obtained in mice models of lymphoma after the treatment with hybrid liposomes without drugs in vivo. The results in this study should support the prolonged survival for patients with lymphoma in clinical applications.

Keywords: Liposome; Lymphoma; Chemotherapy; Apoptosis

1. Introduction

Liposomes are closed vesicles that are formed when phospholipids (constituents of biological membranes) are dispersed in water at relatively low concentrations. Since Bangham et al. discovered liposomes in 1965 (Bangham et al., 1965), these liposomes have been studied for chemical and medical applications. Especially, liposomes have contributed significantly to drug delivery, as well as analysis of cellular function, due to their mimicry of biological membranes and closed properties (Graybill et al., 1982; Gabizon et al., 1994). It is well known that liposomes are used as drug carriers: examples are antitumor agents, hormones, and immunomodulation (Papahadjopoulos and Vail, 1978; Lopez-Berestein and Fidler, 1989). On the other hand, we have recently produced hybrid liposomes composed of vesicular and micellar molecules (Ueoka et al., 1985); they are free from any contamination with organic solvents and remain stable for longer periods. The physical properties of these liposomes such as size, membrane fluidity, phase transition temperature, and hydrophobicity can be controlled by changing the constituents and compositional ratios of hybrid liposomes. In the course of our study on hybrid liposomes, the following interesting results were obtained. (a) Stereochemical control of the enantioselective hydrolysis of amino acid esters could be established by temperature regulation and changing the composition of hybrid liposomes (Ueoka et al., 1988). (b) Inhibitory effects of hybrid liposomes including antitumor drugs (Kitamura et al., 1996) or sugar surfactants (Matsumoto et al., 2000) have been observed on the growth of tumor cells in vitro and in vivo. (c) High inhibitory effects on the growth of leukemia (Matsumoto et al., 2005) and lung carcinoma (Iwamoto et al., 2005) cells in vitro along with the induction of apoptosis using hybrid liposomes have been obtained without using drugs.

In this study, we examined the therapeutic effects of hybrid liposomes on the growth of lymphoma (MOLT-4 or RAJI) in vitro and in vivo using mice models of lymphoma. Furthermore, toxicity of hybrid liposomes was examined using normal rats in vivo.

2. Materials and methods

2.1. Preparation of hybrid liposomes

Hybrid liposomes were prepared by dissolving 95 mol% L-α-dimyristoylphosphatidylcholine (DMPC) and 5 mol% polyoxyethylenedodecyl ether ($C_{12}(EO)_n$) in 5% glucose

Abbreviations: TUNEL, terminal deoxynucleotidyl tranferase-mediated dUTP-biotin nick end labeling; SCID, severe combined immunodeficiency

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solution with sonication (VS-N300, VELVO-CLEAR, Japan) at 45 °C with 300 W, and filtered with a 0.20 μm filter. DMPC (purity > 99%) was obtained from NOF Co. (Japan). $C_{12}(EO)_{21}$ and $C_{12}(EO)_{25}$ are known to be drugs of the Japanese Pharmacopeia and were obtained from Nikko Chemicals Co. Ltd. (Japan).

2.2. Cell culture

Human B lymphoma (RAJI) cell lines and T lymphoma (MOLT-4) cell lines were obtained from RIKEN Cell Bank (Japan). Cells were cultured in RPMI-1640 medium (Gibco BRL, U.S.A.). The media was supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories Inc., U.S.A.) and antibiotics (100 units/ml penicillin and 50 μg/ml streptomycin). The cells were cultured in 5% CO_2 humidified incubator at 37 °C.

2.3. Assessment of inhibition of hybrid liposomes in vitro

Fifty percent inhibitory concentration (IC_{50}) on the growth of tumor cells was determined on the basis of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] assay (Cell Counting Kit-8, Dojindo Laboratories, Japan) (Ishiyama et al., 1993). Cells (1.0×10^5 cells/ml) were seeded in 96-well plate and cultured in an 5% CO_2 humidified incubator at 37 °C for 24 h. Cells were treated with DMPC or hybrid liposomes and were cultured for 48 h. WST-8 solution was added and incubated for 3 h. Absorbance at wavelength of 450 nm was measured by spectrophotometer (E_{max}, Molecular Devices Co., U.S.A.). The inhibitory effects of hybrid liposomes on the growth of tumor cells were evaluated by $A_{mean}/A_{control}$, where A_{mean} and $A_{control}$ denote the absorbance of water-soluble formazan, in the presence and absence of hybrid liposomes, respectively.

2.4. Dynamic light scattering measurement

Apparent mean hydrodynamic diameters (d_{hy}) of hybrid liposomes were measured using light scattering spectrometer (ELS-8000, Otsuka Electronics Co. Ltd., Japan) with He–Ne laser light source (633 nm). The diameter was calculated by Stokes–Einstein equation (Eq. (1)), where κ is the Boltzmann constant, T the absolute temperature, η the viscosity and D is the diffusion coefficient:

$$d_{hy} = (\kappa T)/(3\pi\eta D) \quad (1)$$

2.5. Membrane fluidity

Membrane fluidity of hybrid liposomes were measured using spectrophotofluorometer (F-2000; HITACHI) on the basis of the fluorescence depolarization method. The fluorescence polarization (P) of DPH (1,6-diphenyl-1,3,5-hexatriene, Nacalai tesque, Japan) and tma-DPH [1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene iodide, Dojindo Laboratories, Japan] were measured after sonication of the sample solutions. P values were calculated by Eq. (2), where I is the fluorescence intensity originated from DPH placed into the hydrophobic domain and tma-DPH placed in hydrophobic domain near the membrane surface (Prendergast et al., 1981). The subscripts v and h refer to the orientations, vertical and horizossntal, respectively, for the excitation and analyzer polarizers in this sequence: e.g., I_{vh} indicates the fluorescence intensity measured with a vertical excitation polarizer and horizontal analyzer polarizer. C_f is the grating correction factor, given by I_{hv}/I_{hh}:

$$P = (I_{vv} - C_f I_{vh})/(I_{vv} + C_f I_{vh}) \quad (2)$$

2.6. TUNEL method

Detection of apoptotic cells was performed by TUNEL method using an in situ cell death detection kit (Roche Diagnostics K.K., Switzerland). Sample solutions were added to cell suspension (6.0×10^6 cells) and cultured for 48 h. Concentrations of DMPC liposomes and hybrid liposomes composed of DMPC and $C_{12}(EO)_n$ in the medium were [DMPC] = 0.45 mM and [$C_{12}(EO)_n$] = 0.024 mM. The medium including the dead cells was centrifuged and the cells were fixed with 4% paraformaldehyde solution, and then processed for TUNEL according to the manufacturer's instructions. The stained cells were observed using confocal laser microscope (TCS-SP, Leica Microsystem, Germany) with a 488 nm Ar laser line (detection, 515–565 nm).

2.7. Therapeutic effects of hybrid liposomes in vivo

Female SCID mice (C.B-17/Icr-scid) were obtained from CLEA Japan, Inc. In the primary screening, tumor cells were introduced into the peritoneal cavity, and the test agents were administered intraperitoneally as described in the previous paper (Kanno et al., 1998). The mice were randomly grouped on the basis of body weight on the day of tumor cell inoculation using the stratified randomization method. Number of mice was six in each group. RAJI cells (5.0×10^6 cells) were intraperitoneally injected into the SCID mice. Hybrid liposomes were intraperitoneally administered once each day for 21 days after RAJI cells were intraperitoneally inoculated. The median life span was calculated using the equation of (median survival days after treatment)/(median survival days of control group) × 100.

2.8. Assessment of toxicity in vivo

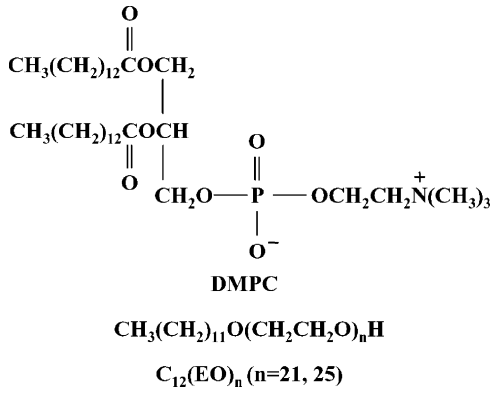
Male rats (Wistar) were obtained from KYUDO Co. LTD. Rats were randomly grouped on the basis of body weight using the stratified randomization method. Number of rats was four in each group. Hybrid liposomes were intravenously administered into the caudal vein of rats once a day for a week. The rats were weighed during the experiment period. The blood was collected from the abdominal aorta after anesthetize with ether. White blood cells (WBC) and red blood cells (RBC) were counted using multiple automatic blood cell county device (F-500, Sysmex Co., Japan). Glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and phospholipids (PL)

were measured using dry chemistry systems (DRICHEM 3500V, FUJIFILM Co. Ltd., Japan). Organs (heart, thymes, lung, liver, spleen, and kidney) were weighed after anatomizing the rats.

3. Results and discussion

3.1. Physical properties of hybrid liposomes

Physical properties of hybrid liposomes composed of L-α-dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene-dodecyl ether ($C_{12}(EO)_n$, $n=21$ or 25) were examined. The diameter of hybrid liposomes was measured using dynamic light scattering spectrometer. The results are shown in Fig. 1. Clear solutions of hybrid liposomes having hydrodynamic diameter of 80–100 nm with narrow range of size distribution could be maintained over 3 weeks. On the other hand, large and unstable liposomes having hydrodynamic diameter of 220–320 nm were obtained for the DMPC liposomes, which had wide range of size distribution. Membrane fluidity of hybrid liposomes was measured on the basis of fluorescence depolarization method using a fluorescent probe. Fluorescence polarizations (P) are shown in Fig. 2. The fluidity of the hydrophobic domain of hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ were clearly larger than that of the DMPC liposomes as shown in Fig. 2(A).



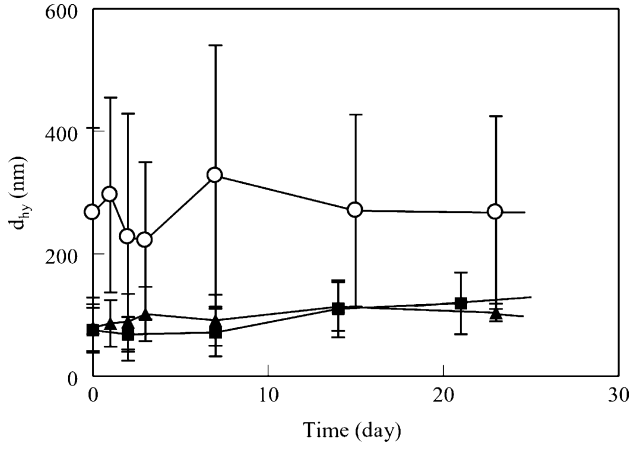


Fig. 1. Time courses of d_{hy} change for hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-n). Error bar indicates size distribution. (◯) DMPC, (■) HL-21, (▲) HL-25, [DMPC] = 1.0×10^{-3} M.

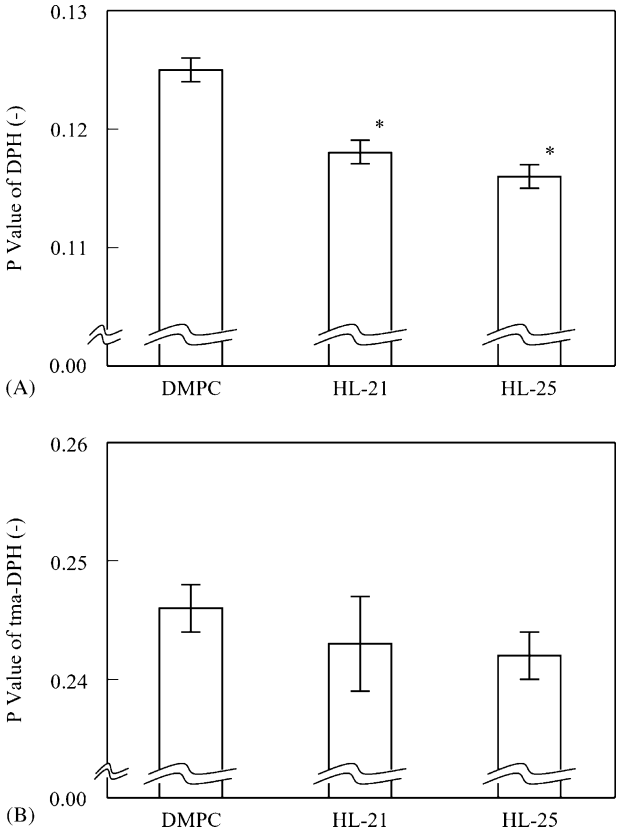


Fig. 2. P values of DPH (A) and tma-DPH (B) in hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-n). Data represent the mean ± S.D. $^*P<0.01$, compared with DMPC liposomes.

3.2. Inhibitory effects of hybrid liposomes on the growth of MOLT-4 and RAJI cells

We examined 50% inhibitory concentration of hybrid liposomes (DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$) on the growth of MOLT-4 and RAJI cells in vitro. The results are shown in Fig. 3. The IC_{50} values of hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ were smaller than those of the DMPC liposomes on the growth of MOLT-4 cells, although the IC_{50} of hybrid liposomes and DMPC liposomes were almost the same in the case of RAJI cells.

Fluorescence micrographs of MOLT-4 and RAJI cells treated with hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ on the basis of TUNEL method are shown Fig. 4. Interestingly, MOLT-4 cells were dyed in green after adding hybrid liposomes (HL-21: 16%, HL-25: 26%), indicating that hybrid liposomes induced apoptosis for MOLT-4 cells, although cells were not dyed using the DMPC liposomes. The apoptotic cell percentages were calculated using the equation of (number of cells dyed in green)/(number of all cells) × 100. On the other hand, RAJI cells were dyed in green after adding both hybrid liposomes (HL-21: 11%, HL-25: 17%) and the DMPC liposomes (3%). The specific apoptosis induced by hybrid liposomes for MOLT-4 cells was in harmony with the smaller IC_{50} values of hybrid liposomes.

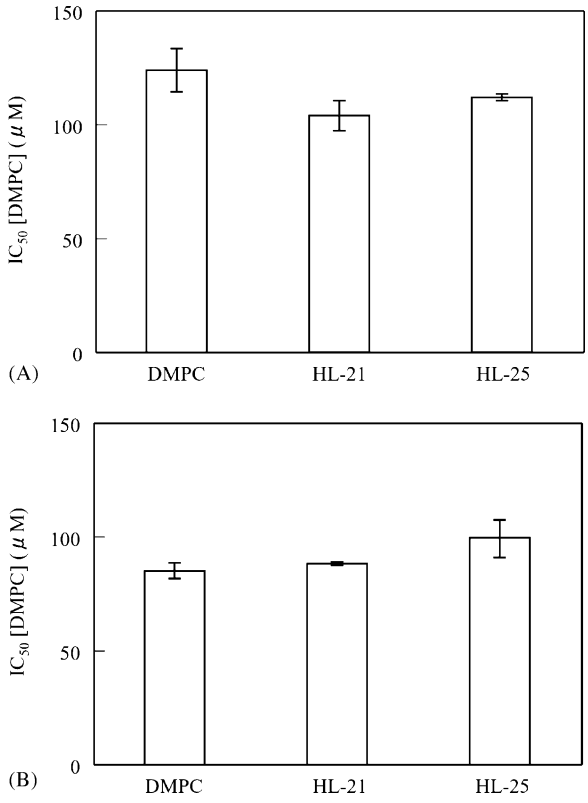


Fig. 3. 50% inhibitory concentration (IC_{50}) of hybrid liposomes composed of 95 mol% DMPC and 5 mol% $C_{12}(EO)_n$ (HL-*n*) on the growth of MOLT-4 cells (A) and RAJI cells (B). Data represent the mean ± S.D.

Table 1
Hematological findings in normal rats treated intravenously with hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-*n*) for 7 days

Sample	Dose (mg/kg)		RBC ($\times 10^4$)	WBC ($\times 10^2$)
	DMPC	$C_{12}(EO)_n$		
Control	–	–	684 ± 27	65 ± 9
HL-21	67.8	5.84	619 ± 93	66 ± 20
HL-21	271	23.3	548 ± 81	164 ± 103
HL-25	67.8	6.76	648 ± 60	52 ± 19
HL-25	271	27.0	493 ± 44	245 ± 142

Data represent the mean ± S.D. Number of rats was four in each group. The DMPC dose of the hybrid liposomes was 49.5 mg/kg for clinical application.

3.3. Toxicity of hybrid liposomes in vivo

Safety tests were examined using normal rats treated intravenously with the hybrid liposomes of DMPC/5mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$. No weight loss was observed in the rats after being treated intravenously with hybrid liposomes at DMPC doses of 67.8 and 271 mg/kg. Hematological tests were examined and results were shown in Table 1. Low doses of hybrid liposomes produced no abnormal findings, although red blood cell count slightly decreased and white blood cell count increased in rats after the treatment with the high doses of hybrid liposomes. Microscopic observations revealed that lymphocytes increased in rats after the treatment with high doses of hybrid liposomes.

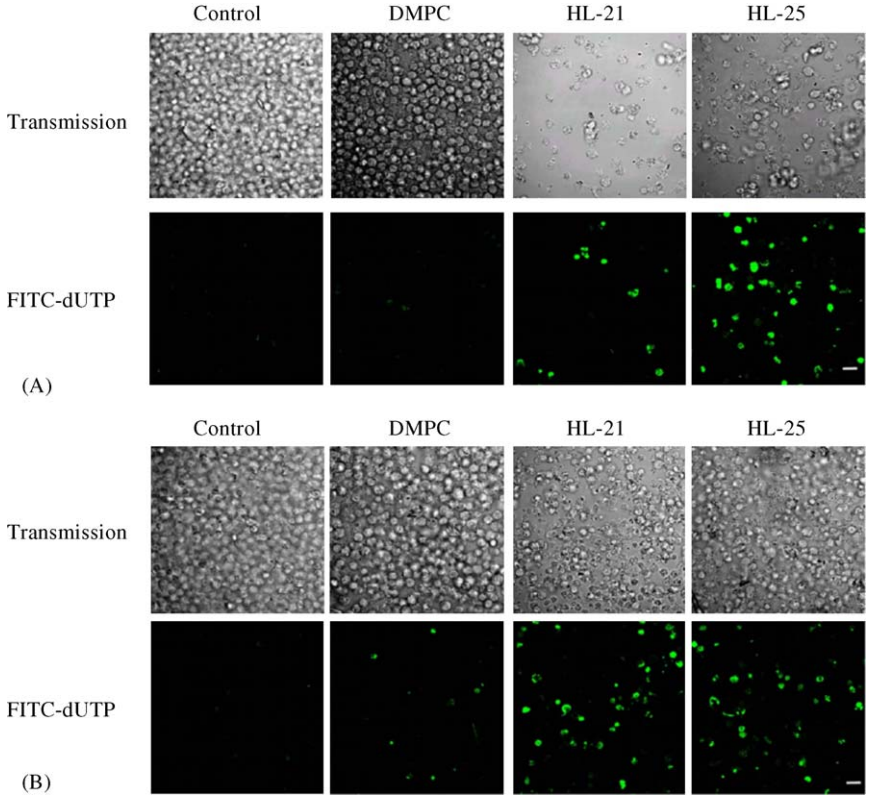


Fig. 4. Fluorescence micrographs of MOLT-4 cells (A) and RAJI cells (B) treated with hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-*n*) for 24 h using TUNEL method. Scale bar 20 μm, [DMPC] = 0.45 mM, [$C_{12}(EO)_n$] = 0.024 mM.

Table 2
Biochemical findings in normal rats treated intravenously with hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-*n*) for 7 days

Sample	Dose (mg/kg)		GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	BUN (mg/dl)	PL (mg/dl)
	DMPC	$C_{12}(EO)_n$					
Control	–	–	101 ± 16	21 ± 3	869 ± 183	14.3 ± 1.4	160 ± 18
HL-21	67.8	5.84	104 ± 17	20 ± 2	765 ± 68	12.1 ± 1.5	146 ± 44
HL-21	271	23.3	97 ± 18	18 ± 2	820 ± 100	15.2 ± 1.1	223 ± 39
HL-25	67.8	6.76	80 ± 10	21 ± 2	761 ± 166	13.6 ± 1.9	178 ± 63
HL-25	271	27.0	89 ± 16	22 ± 4	693 ± 224	15.8 ± 3.0	158 ± 14

Data represent the mean ± S.D. Number of rats was four in each group. The DMPC dose of the hybrid liposomes was 49.5 mg/kg for clinical application.

Table 3
Relative organ weight in normal rats treated intravenously with hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_n$ (HL-*n*) for 7 days

Sample	Dose (mg/kg)		Relative organ weight (g/100 g body weight)					
	DMPC	$C_{12}(EO)_n$	Heart	Thymus	Lung	Liver	Spleen	Kidney
Control	–	–	0.38 ± 0.04	0.28 ± 0.02	0.51 ± 0.04	3.60 ± 0.46	0.28 ± 0.06	0.79 ± 0.03
HL-21	67.8	5.84	0.40 ± 0.02	0.30 ± 0.03	0.52 ± 0.02	3.54 ± 0.06	0.29 ± 0.02	0.87 ± 0.03
HL-21	271	23.3	0.38 ± 0.03	0.26 ± 0.05	0.55 ± 0.06	3.43 ± 0.14	0.68 ± 0.11	0.78 ± 0.08
HL-25	67.8	6.76	0.37 ± 0.01	0.32 ± 0.01	0.51 ± 0.03	3.25 ± 0.13	0.33 ± 0.09	0.79 ± 0.08
HL-25	271	27.0	0.40 ± 0.02	0.25 ± 0.07	0.54 ± 0.02	3.34 ± 0.15	0.93 ± 0.09	0.77 ± 0.06

Data represent the mean ± S.D. Number of rats was four in each group. The DMPC dose of the hybrid liposomes was 49.5 mg/kg for clinical application.

Biochemical findings in rats treated intravenously with hybrid liposomes are shown in Table 2. No difference between the control group and the treatment groups was observed except for an increase of white blood cells at high dose condition, suggesting that the hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ should be safely metabolised in rats.

Relative organ weight in rats treated intravenously with hybrid liposomes are shown in Table 3. No difference was observed between the control group and the treatment groups in the heart, thymus, lung, liver and kidney, although an increase in the weight of the spleen after the treatment with the high doses of hybrid liposomes occurred. These observations were almost the same after the treatment with a fatty emulsion (Reimold, 1979), so the rats could recover in a short time span after the intravenous injection of the hybrid liposomes.

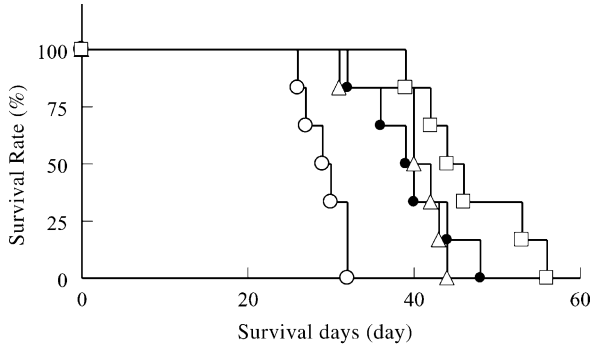


Fig. 5. Survival curves of mice treated with hybrid liposomes after the inoculation of RAJI cells. (◯) control, (●) DMPC (136 mg/kg), (△) HL-21 (DMPC 136 mg/kg, $C_{12}(EO)_{21}$ 11.7 mg/kg), (□) HL-25 (DMPC 136 mg/kg, $C_{12}(EO)_{25}$ 13.5 mg/kg).

3.4. Therapeutic effects of hybrid liposomes in vivo

We examined the therapeutic effects of hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ using mice models of lymphoma after the inoculation of RAJI cells in vivo. The results are shown in Fig. 5. The median survival time was 30.0 days in the control group. It is noteworthy that significantly prolonged survival (140 and 160%) was obtained in mice treated with the hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and the DMPC liposomes, and the hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{25}$, respectively. However, hydrodynamic diameter of hybrid liposomes and the DMPC liposomes are 80–100 and 220–320 nm, respectively, so hybrid liposomes could avoid the reticular endothelial system after intravenous administration. Clinical application of hybrid liposomes for patients with lymphoma was performed after being passed by the Committee of Bioethics (Ueoka et al., 2002). In this study, a mouse model of lymphoma was successfully established and prolonged survival was obtained by the administration of hybrid liposomes in vivo. This result supports the observations evident in patients with lymphoma who achieved a prolonged survival rate through the administration of hybrid liposomes.

4. Conclusion

We clearly demonstrated that a mouse model of lymphoma was established and prolonged survival was obtained by the administration of hybrid liposomes of DMPC/5 mol% $C_{12}(EO)_{21}$ and DMPC/5 mol% $C_{12}(EO)_{25}$ in vivo. The noteworthy aspects are as follows. (a) Clear solution of hybrid liposomes having hydrodynamic diameter of 80–100 nm could be maintained over 3 weeks. (b) Hybrid liposomes induced apoptosis

for human lymphoma cells. (c) No toxicity was observed in the rats after intravenously injecting hybrid liposomes. (d) Prolonged survival was obtained in mice models of lymphoma after the treatment with hybrid liposomes. The results in this study should support the prolonged survival for patients with lymphoma in clinical applications.

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References

Bangham, A.D., Sandish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., Barenholz, Y., 1994. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res. 54, 987–992.

Graybill, J.R., Craven, P.C., Taylor, R.L., Williams, D.M., Magee, W.E., 1982. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J. Infect. Dis. 145, 748–752.

Ishiyama, M., Shiga, M., Sasamoto, K., Mizoguchi, M., 1993. A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. Chem. Pharm. Bull. 41, 1118–1122.

Iwamoto, Y., Matsumoto, Y., Ueoka, R., 2005. Induction of apoptosis of human lung carcinoma cells by hybrid liposomes containing polyoxyethylenedodecyl ether. Int. J. Pharm. 292, 232–239.

Kanno, A., Kodama, R., Terada, Y., Matsumoto, Y., Ueoka, R., 1998. Chemotherapy with hybrid liposomes for cancer: an experimental study. Drug Del. Syst. 13, 101–105.

Kitamura, I., Kochi, M., Matusmoto, Y., Ueoka, R., Kuratsu, J., Ushio, Y., 1996. Intrathecal chemotherapy with 1 3-bis(2-chloroethyl)-1-nitrosourea encapsulated into hybrid liposomes for meningeal gliomatosis: an experimental study. Cancer Res. 56, 3986–3992.

Lopez-Berestein, G., Fidler, I. (Eds.), 1989. Liposomes in the Therapy of Infectious Disease and Cancer. Alan. R. Liss. Inc., New York.

Matsumoto, Y., Kato, T., Suzuki, H., Hirose, H., Naiki, Y., Hirashima, M., Ueoka, R., 2000. Highly specific inhibitory effects of three-component hybrid liposomes including sugar surfactants on the growth of glioma cells. Bioorg. Med. Chem. Lett. 10, 2617–2619.

Matsumoto, Y., Iwamoto, Y., Matsushita, T., Ueoka, R., 2005. A novel mechanism of hybrid liposomes-induced apoptosis in human tumor cells. Int. J. Cancer 115, 377–382.

Papahadjopoulos, D., Vail, W.T., 1978. Incorporation of macromolecules within large unilamellar vesicles. Ann. N.Y. Acad. Sci. 308, 259–267.

Prendergast, F.G., Haugland, R.P., Callahan, P.J., 1981. 1-[4(Trimethylamino)-phenyl]-6-phenylhexa-1,3,5-triene: synthesis, properties, and use as a fluorescence probe of lipid bilayers. Biochemistry 20, 7333–7338.

Reimold, E.W., 1979. Studies of the toxicity of an intravenous fat emulsion. Hematologic changes and survival after administration of a soybean oil (FE-S15) in beagles. J. Parenter. Enteral. Nutr. 3, 328–334.

Ueoka, R., Moss, R.A., Swarup, S., Matsumoto, Y., Strauss, G., Murakami, Y., 1985. Extraordinary micellar enantioselectivity coupled to altered aggregate structure. J. Am. Chem. Soc. 107, 2185–2186.

Ueoka, R., Matsumoto, Y., Moss, R.A., Swarup, S., Sugii, A., Harada, J., Kikuchi, Y., Murakami, Y., 1988. Membrane matrix for the hydrolysis of amino acid esters with marked enantioselectivity. J. Am. Chem. Soc. 110, 1588–1595.

Ueoka, R., Matsumoto, Y., Ichihara, H., Kiyokawa, T., 2002. Chemotherapy with hybrid liposomes composed of dimyristoylphosphatidylcholine and polyoxyethylenealkyl ether without drugs. Am. Chem. Soc. Books, 177–189.